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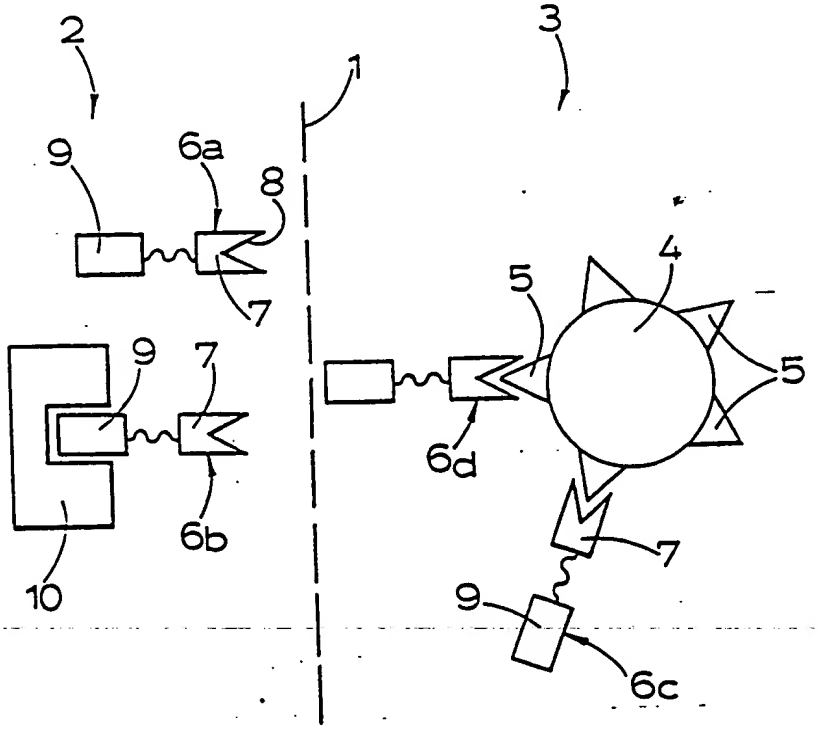
(54) Title: TUMOUR-TARGETING AGENTS AND A METHOD OF TARGETING TUMOURS

(57) Abstract

An agent for use in targeting tumour cells comprises a compound (6) of a mean molecular weight of no more than about 70,000, and preferably of considerably lower molecular weight; it is thus able to pass relatively readily from the bloodstream into the extra-cellular fluid. The compound is able to become reversibly bound to characteristic antigens (5) on tumour cells (4) and to this end may include fragments (7) of antibodies. The compound also includes a ligand (9) that can become reversibly bound to a binding protein (10) naturally occurring in the bloodstream or introduced into the bloodstream. The complex formed by the compound and binding protein is eliminated from the body only relatively slowly and thus provides a continuing source of the compound for attachment to the tumour antigens. The compound may be radioactive or include a chemotherapeutic constituent. When it is desired to flush the compound from the body, an unreactive substance that competes with the binding protein is introduced into the bloodstream at relatively high concentrations, thus displacing the compound from the binding protein and enabling it to be eliminated by the kidneys.



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TUMOUR-TARGETING AGENTS AND A METHOD OF TARGETING TUMOURS

This invention relates to tumour-targeting agents and a method of targeting tumours.

A known characteristic of many tumour cells is that they have on their surfaces numerous tumour antigens which form reactive sites to which antibodies can become bound. About 90% of tumour-types have identifiable antigens, and it seems likely that other have antigens that will in due course be identified. An obvious approach to the targeting of tumour cells is to employ antibodies that are complementary to the antigens. As the average molecular weight of an antibody is relatively large, however, individual antibody molecules pass only relatively rarely from the bloodstream, through the walls of the blood vessels, into contact with tumour cells. Moreover, as the binding reaction between antibodies and the complementary sites on tumour cells is a reversible reaction, it is found that in normal circumstances the concentration of antibodies in the neighbourhood of a tumour cell is insufficient to enable antibodies to congregate markedly on tumour cells.

Also, certain tumour cells can be targeted by other compounds, those compounds being of relatively low molecular weight. While such compounds can pass relatively easily from the bloodstream to the extra-cellular fluid (ECF), they are rapidly eliminated in the kidneys so that the concentration of those compounds bound to tumour antigens tends to fall rapidly from a maximum value.

An aim of the present invention is to provide tumour-targeting agents that avoid the problems outlined above.

From a first aspect the present invention consists in a tumour-targeting agent comprising a compound of mean molecular weight no more than about 70,000 that can become bound to a characteristic reactive site on a tumour cell, and that has a ligand comprising an attachment portion capable of becoming reversibly bound to a complementary binding protein so as to form a complex with said binding protein.

The compound preferably incorporates a fragment of an antibody, that fragment including an antigen-binding portion.

Antigen-binding sites are known to be present in the F_{ab} parts of antibodies, and these parts of antibodies can conveniently be used as the source of antigen-binding portions in compound in accordance with the present invention.

The arrangement is such that when an appropriate quantity of a compound in accordance with the invention is introduced into the bloodstream of a living human being or animal, and an appropriate binding protein is also present, the ligand becomes reversibly bound to the binding protein if it has not already become bound to it. The average molecular weight of the resultant complex is sufficiently large to ensure that the compound is not flushed rapidly from the bloodstream by the kidneys, but the average molecular weight of the compound itself is sufficiently low that, in use, when the compound is released from the binding protein it can escape through the walls of the blood vessels into

the ECF at a sufficient rate and in sufficient quantities to enable it to become preferentially bound to any accessible tumour cells that may be present in the human being or animal, so that at least some of such tumour cells become characterised by the fact that each such cell has many more molecules of the compound bound to it than there are molecules of the compound bound to an average, normal cell, or the concentration of the compound bound to at least some of the tumour cells is significantly greater than the concentration elsewhere in the human being or animal.

The invention takes advantage of the fact that the rate at which a substance can enter the ECF from the bloodstream by passing through the walls of the blood vessels is dependent on its molecular weight: the lower the molecular weight of a substance, the higher the rate at which the substance can pass through the walls.

In general, therefore, it is necessary or desirable to seek a tumour-targeting agent of relatively low molecular weight so that it can pass from the bloodstream, through the walls of the blood vessels and into the ECF, at a sufficient rate and in sufficient quantities to enable the concentration of the agent on tumour cells to be much greater than its concentration on normal cells, or elsewhere in the system. The present invention provides an agent of that kind. If an agent of relatively low molecular weight is introduced into the bloodstream, without being bound to a binding protein, such an agent tends to be removed so rapidly from the bloodstream by the action of the kidneys that once a maximum concentration of agent bound to tumour antigens has been reached, the concentration falls off again relatively rapidly.

It will be appreciated that the binding that occurs between a compound embodying the present invention and a characteristic reactive site on a tumour cell is reversible. The dynamics of that bond are governed by an association rate constant k_a and a dissociation rate constant k_d. If a tumour-targeting agent of relatively low molecular weight were employed without the use of a complementary binding protein, the proportion of the compound introduced into the bloodstream and bound to the tumour antigens would rapidly reach a maximum value but would relatively quickly become reduced again.

The present invention can largely overcome those problems by providing an agent that includes a ligand capable of becoming reversibly bound to a binding protein. The ligand is such that the average molecular weight of the complex formed by the compound and the binding protein is sufficiently great to prevent the complex being rapidly removed from the bloodstream by the kidneys. There is therefore an opportunity for the complex to become distributed throughout the bloodstream. Further, the equilibrium constant controlling the binding of the compound to the binding protein is such that sufficient compound is released in the bloodstream to ensure that a significant proportion of the agent passes through the walls of the blood vessels into the ECF before it is removed from the bloodstream by the kidneys. This enables the compound to reach any tumour cells in sufficient quantities and at a sufficient rate to enable it to congregate markedly on those cells rather than on normal cells or elsewhere in the system. Moreover, the parameters can be so selected that the proportion of the introduced compound bound to the tumour antigens remains

relatively high so that the compound has a longer time in which to become effective.

A compound embodying the present invention can be used in any of a number of different ways. It may, for example, incorporate radioactive atoms such as technetium-99, iodine-131 or yttrium-90. These may, serve either to enable tumour cells to be targeted and detected photographically or to irradiate tumour cells to which the compound becomes bound. Iodine-131, for example, may be incorporated for the purpose of irradiating tumour cells. Alternatively, or in addition, the compound may include one or more additional radicals which can aid in enabling the tumour cells to be located or to be destroyed or otherwise rendered ineffective. These may well be based on known chemotherapeutic agents such as methotrexate or cyclophosphamide. Such a radical may, for example, attack a tumour cell to which the compound becomes bound. Alternatively a radical may be used that can provide an anchorage to which a secondary agent, for use in locating or attacking the cell, can be chemically attached in situ.

While it may be possible for a compound in accordance with the invention to employ the whole of a F_{ab} monomer of an antibody as an antibody fragment, it is preferred to employ only a part of an arm as such a fragment. An entire arm may have a molecular weight of about 50,000, whereas it is preferred to use a fragment with a molecular weight of no more than about 10,000. Indeed, it may well prove advantageous to employ a fragment with a molecular weight of about 5,000 or less, so that the whole compound has a molecular weight of about 5,000. The necessary cleavage of the antibody and the extraction and

purification of the fragments incorporating antigen-binding portions can be effected by conventional techniques.

A convenient ligand is thyroxine or a non-functional thyroxine analogue, which in use becomes reversibly attached to a binding protein of relatively high molecular weight that is present in the bloodstream, namely thyroxine binding globulin.

Conventional chemical techniques can be used to join together, directly or indirectly, the antigen-binding portion and the ligand to form a compound that is characteristic of the present invention.

The binding protein to which the compound becomes reversibly attached may be constituted by a protein such as the globulin mentioned above, which is naturally present in the blood. Alternatively the binding protein or additional binding protein may be introduced into the bloodstream. The binding protein may be selected or created so as to enable it to become reversibly bound to an attachment portion of any desired constitution. If desired, the binding protein may consist of IgM, which comprises an assembly of five Y-shaped antibody constituents.

If desired, the agent and the binding protein may be combined <u>in vitro</u> to form a complex that is then introduced into the bloodstream.

Therefore, from a second aspect the present invention consists in a tumour-locating agent comprising a complex constituting a compound in

accordance with the first aspect of the present invention and a complementary binding protein.

The dynamics of the reversible attachment of the compound and the binding protein are governed by an equilibrium constant. In general it is preferred to provide a system in which the equilibrium constant controlling the binding of the compound to the binding protein is several orders of magnitude less than the equilibrium controlling the binding of the compound to a reactive site on a tumour cell, so that the compound becomes bound to the reactive site much more strongly than it becomes bound to the binding protein.

The dynamics of the system also depend to a significant extent on the rate at which the walls of the blood vessels allow molecules of the compound to pass through them while preventing or hindering the flow of molecules of the binding protein. The rate at which any material diffuses from the blood vessels into the ECF is determined principally by the concentration of the material in the bloodstream and by a diffusion constant characteristic of that material. Preferably the system is such that the diffusion constant controlling the rate of flow of the unbound compound through the walls of the blood vessels is at least ten times greater than the diffusion constant controlling the rate of flow of the binding protein through the walls of the blood vessels, so that there is preferential passage of the compound through the walls of the blood vessels.

In some circumstances it may be necessary or desirable to introduce an agent embodying the present invention into the bloodstream, to allow a period of time to pass sufficient to enable an adequate quantity

of the compound to become bound to any tumour cells present, and then to remove from the bloodstream at least a substantial part of any of the compound remaining in the bloodstream.

Removal of the compound from the bloodstream can be effected by introducing into the bloodstream a substance that is capable of competing with the compound that is characteristic of the present invention for becoming bound to the complementary binding protein. As the bond between the compound and the binding protein is reversible, individual molecules of the compound frequently become released from the binding protein and then become re-attached. If the competing substance is present, however, some molecules of that substance become attached to the binding protein in place of the molecules of the compound, so that the concentration of unattached molecules of the compound is increased. This is a direct consequence of the law of mass action. As a result of this, the introduction of the substance into the bloodstream brings about an increase in the rate at which the compound is removed from the bloodstream by the kidneys. In addition there is a temporary increase in the concentration of the compound in the ECF, so that there may be an increase in the take-up of the compound by any tumour cells present. Moreover, an increase in concentration of the competing substance leads to an increase in the rate at which the compound is eliminated from the bloodstream. To hasten these effects, the substance may, for example, be introduced into the bloodstream at a concentration many times greater than that at which the compound is present, for example at a concentration ten times or a hundred times greater than that at which the compound is present.

Unattached compound in the ECF diffuses back into the blood vessels and is also eliminated.

Several advantages can result from the introduction of a competing substance in this manner. First, it enables radioactive or toxic materials to be removed relatively rapidly from the body after a predetermined period and thus prevents the body being subjected to those materials for a longer period than is necessary. Consequently it enables larger concentrations of those materials to be introduced initially than would otherwise be the case, for the period of time during which the body is subjected to those materials is only a relatively short one, and the body is able to withstand the ill effects of those materials in those relatively large concentrations for that relatively short period. Secondly, it is known that at least some tumour cells continuously form replacement antigens. Consequently, a compound characteristic of the invention may initially target antigens on a tumour cell satisfactorily, but the compound is then likely to be drawn into the cell, and fresh antigens are likely to be generated in the cell and be exposed on the surface. The upsurge in the concentration of free compound that occurs when a competing substance is introduced into the bloodstream may thus assist in subjecting the exposed antigens to a second attack by the compound. Ingestion of the compound by the cell may lead to destruction of the cell, this being hastened by the additional concentration of the compound. Thirdly, when the compound incorporates a radioactive marker that serves to locate a tumour, the addition of a competing substance of the kind described leads to a temporary increase in the concentration of the compound on the tumour cells and a simultaneous reduction in the

concentration of compound elsewhere in the body, thereby reducing the background radiation and improving the accuracy of the location.

The competing substance is preferably such that it has no toxic or deleterious effects on the patient or subject into whose bloodstream it is introduced. Thyroxine may be an appropriate substance when the ligand comprises or is based on thyroxine. In general the competing substance will have an attachment portion similar to that constituting part of the ligand which in turn constitutes part of the compound that is characteristic of the present invention.

From a third aspect the present invention consists in a method of employing a tumour-targeting agent in accordance with the first aspect of the present invention, in which method a quantity of the agent is introduced into the bloodstream of a living human being or animal.

In a preferred method, a period of time is allowed to elapse sufficient to enable at least some of any tumour cells present to become characterised by the fact that each such cell has many more molecules of the compound bound to it than there are molecules of the compound bound to an average normal cell, or the concentration of the compound bound to at least some of tumour cells is significantly greater than the concentration elsewhere in the human being or animal.

Preferably, after that period of time, a substance is introduced into the bloodstream which competes with said compound for becoming bound to said complementary binding protein, whereby the rate at which said

compound is eliminated from the bloodstream is increased.

The substance preferably has an attachment portion similar to the attachment portion that constitutes part of the compound.

Example

In one particular type of embodiment of the present invention a compound is made which incorporates fragments of monoclonal or polyclonal antibodies that have been highly characterised. The antibodies are selected by hyperimmunisation of mice or other animals and by testing the resultant antibodies against known tumour antigens. The selected antibody is fragmented by subjecting the antibody to limited digestion by proteolytic enzymes. The resultant fragments are separated according to size by size-separation column chromatography.

The selected fragments incorporate antigen-binding portions that bind reversibly with the tumour antigens. The fragments are then attached to a ligand consisting of a non-functional thyroxine analogue of a kind having no pharmacological properties. The thyroxine analogue contains radioactive iodine-131. The antibody fragments and the ligand are joined together by any appropriate chemical method such as the periodate reaction or by using gluteraldehyde. The agent thus formed has an average molecular weight of about 5000.

In use about 50 to 100 mg of the agent is injected intraveneously into a human patient during a period of a few minutes. That part of the agent comprising the

thyroxine analogue constitutes an attachment portion which becomes reversibly bound to thyroxine binding globulin naturally occurring in the blood. The resulting complex has an average molecular weight greater than 70,000.

In the accompanying drawings:-

Figure 1 is a diagram illustrating one form of the present invention, and

Figures 2 to 6 are graphs illustrating the present invention.

In Figure 1 there is shown diagrammatically a wall 1 of a bloodvessel which divides the interior 2 of the bloodvessel from the ECF 3. A tumour cell 4 in the ECF has characteristic antigens 5 on its surface. An agent incorporating a compound 6 embodying the invention has been injected into the bloodstream and several molecules 6a, 6b, 6c and 6d of the compound are illustrated. Each molecule of the compound comprises a fragment 7 of an antibody including a binding-portion 8 that can become bound to one of the antigens 5. The agent also includes a ligand 9 which is capable of becoming reversibly bound to a complementary binding protein. The molecule 6b is shown bound to a molecule 10 of such a protein and forming a complex with the binding protein. The complex circulates through the body and by virtue of its relatively large atomic weight is not readily eliminated by the kidneys. Because of that relatively large atomic weight, however, the complex is unable readily to pass through the wall 1. Dissociation of the complex frees the agent 6 and as the agent is of relatively low molecular weight it is able more readily to pass

through the wall 1 into the ECF where it can become attached to the antigens 5. Molecules 6c and 6d are shown as attached to the antigens.

The agent 6 may include radioactive elements or chemotherapeutic agents as described above.

The results of some theoretical calculations are illustrated in the remaining Figures. These are based on a model consisting of two compartments comprising essentially the blood plasma in a human being, occupying the interior 2 of the bloodvessels, and the ECF. A dose of agent in accordance with the invention is introduced into the plasma as a bolus and the compound which is the characteristic component of that agent is progressively eliminated by way of the kidneys. Before that, however, the compound is involved in various reversible activities: it becomes reversibly bound to a binding protein, both in the plasma and in the ECF, it reversibly passes through the walls 1 of the bloodvessels, and from the ECF it becomes reversibly bound to tumour cells and, though only in small concentrations, to normal cells. The binding protein is also able to pass reversibly from the blood plasma through the walls 1 of the bloodvessels into the ECF, though at a rate much less than that at which unbound compound can pass. In the model no account is taken of other effects because it is believed that they are relatively unimportant. The model gives rise to a series of simultaneous differential equations which reflect the changes that take place with time and which can be solved by computer.

In deriving solutions to the equations the following input variables were used: the dose of

compound was 6 x 10^{-10} moles; the plasma volume was 2.5 l; the tumour antigen quantity was 2 x 10^{-10} moles; the tissue receptor concentration was 100 times less than the tumour-antigen concentration; the tumour volume was 10 ml; the normal tissue volume was 12 l; the ECF volume was 12 l; the relationship between molecular radius and the permeability of the walls of blood vessels was based on work published in the following papers:

- Arfors, K.-E., Rutili, G., and Svensjo, E. Microvascular transport of macromolecules in normal and inflammatory conditions. Acta. Physiol. Scand. Suppl., 463: 93-103, 1979.

- Garlick, D. G. and Renkin, E. M. Transport of large molecules from plasma to interstitial fluid and lymph in dogs. Am. J. Physiol., 219: 1595-1605, 1970.

- Pilz, I., Kratky, O., Licht, A., and Sela, M. Shape and volume of fragments Fab' and $F(ab')_2$ of anti-poly(D-alanyl) antibodies in the presence and absence of tetra-D-alanine as determined by small-angle x-ray scattering. Biochemistry, 14: 1326-1333, 1975.

The rate at which the compound becomes bound with tumour antigens and the rate at which the compound becomes dissociated from tumour antigens are based on typical rates at which antibodies become bound and dissociated. The association rate constant k_a may vary between about 10^5 $M^{-1}s^{-1}$ and 10^8 $M^{-1}s^{-1}$, while the dissociation rate constant k_d may vary between $10^{-5}s^{-1}$ and $10^{-3}s^{-1}$. The equilibrium constant K_{eq} is equal to k_a/k_d and varies from 10^8 M^{-1} upwards to 10^{12} M^{-1}

or even more. The equilibrium constant for the binding of the compound to the binding protein was taken as $10^4 \cdot M^{-1}$.

Calculations were made of the tumour content (TC) and the tumour: background uptake ratio (UR) over time. TC equals the number of moles of the agent bound to the tumour antigens at any instant expressed as a percentage of the injected dose, and UR is calculated as the ratio of the concentration of the agent bound to the tumour to the mean concentration elsewhere in the body.

Clearly it is desirable that the UR should reach or at least closely approach a maximum value relatively quickly, for otherwise relatively large quantities of the compound will be dwelling in parts of the body where it is not required and may do harm or, if mildly radioactive, prevent the clear location of a tumour by photographic or other means used for dectecting radiation. Likewise it is desirable for the TC to reach a maximum value relatively quickly and for the rate of reduction from that maximum value to be relatively slow.

Figure 2 shows the predicted effect of changes in molecular weight of an injected compound on UR, in the absence of any binding protein. The horizontal axis represents time in days, and the vertical axis represents UR. The curve 11 represents the use of a whole antibody (molecular weight about 150, 000), the curve 12 represents the use of a $F_{ab'}$ (that is a F_{ab} monomer) fragment (molecular weight about 50,000) and the curve 13 represents the use of a small molecule which can pass rapidly through the walls of bloodvessels. The binding affinity with between the

injected compound and the tumour is taken to be $K_{eq} = 10^{10}\ M^{-1}$.

Figure 3 corresponds to Figure 2 and illustrates the variations in TC, this being expressed as a percentage. Curves 14, 15 and 16 represent the use of whole antibody, $F_{ab'}$ fragments and small molecules respectively.

The graphs show that an increase in the rate of maximisation so UR and TC is achieved by a reduction in the size of the molecules of the substance introduced into the bloodstream.

Other calculations show that, when using an injected substance of small molecule-size, it is desirable for K_{eq} to be relatively large, values above $10^{10}\ M^{-1}$ showing significant maxima, and those of about $10^{12}\ M^{-1}$ showing further increases in the maxima. Calculations also show that for any given value of K_{eq} is desirable for k_d to be as small as possible, as a reduction in k_d tends to lead to a delay in the rate at which both the UR and TC reduce after reaching their maxima. Values of k_d of $10^{-3}\ M^{-1}s^{-1}$ or less are desirable, those of about $10^{-5}\ M^{-1}s^{-1}$ are preferred.

The use of a compound in accordance with the present invention, which is able to form a complex with a binding protein present in the bloodstream tends to lower the peak values of TC and UR but to prolong the period during which the compound remains bound to the tumour antigens. This is illustrated in Figures 4 and 5.

In Figures 4 and 5 the horizontal axis represents time is hours (not days, as in Figures 2 and 3). The vertical axis in Figure 4 represents UR, and that in Figure 5 TC. In Figure 4, curve 17 corresponds to curve 13 of Figure 2 and shows how the uptake ratio of a small molecule varies with time when there is no binding protein present. Curve 18 shows what happens when a binding protein, having an equilibrium constant K_{eq} equal to 10^4 M^{-1}, is present at a concentration of 10^{-4} M plasma concentration; curve 19 is similar but here there is binding protein at a concentration of 10^{-3} M plasma concentration. In Figure 5, curves 20, 21 and 22 show the TC corresponding to curves 17, 18 and 19 respectively.

Figure 6 shows the effect on the TC of the introduction, after some hours, of a substance competing with the binding protein. In this Figure the horizontal axis represents time in hours and the vertical axis represents TC. Curve 23 is the same as curve 20 and shows the change in TC that occurs when no binding protein is present. Curve 24 is the same as curve 22 and shows the effect on the TC of the presence of a binding protein at a concentration of 10^{-3} M plasma concentration. Curve 25 shows the sudden increase in TC that occurs when a competing substance is introduced. The competing substance has the same affinity for the binding protein as has the compound characteristic of the invention, and its concentration is 100 times greater than that of the compound. As described above, the newly introduced substance displaces the compound from the binding protein, with the result that curve 25 is very similar to curve 23 but is displaced in time.

It will be appreciated that when compound is used that irradiates tumour cells or has a toxological effect on tumour cells, the effectiveness of the agent is closely related to the area under the TC curve. By use of a compound which becomes reversibly bound to a binding protein and the subsequent use of a competing substance to displace the compound from the binding protein, it is possible to control the treatment in a desirable manner.

CLAIMS

1. A tumour-targeting agent comprising a compound of mean molecular weight no more than about 70,000 that can become bound to a characteristic reactive site on a tumour cell, and that has a ligand comprising an attachment portion capable of becoming reversibly bound to a complementary binding protein so as to form a complex with said binding protein.

2. A tumour-targeting agent according to claim 1 in which the compound is of mean molecular weight of no more than about 50,000.

3. A tumour-targeting agent according to claim 1 in which the compound is of mean molecular weight of no more than about 10,000.

4. A tumour-targeting agent according to claim 1 in which the compound is of mean molecular weight of no more than about 5,000.

5. A tumour-targeting agent according to any one of claims 1 to 4 in which the compound incorporates a radioactive element.

6. A tumour-targeting agent according to any one of claims 1 to 4 in which the compound incorporates a chemotherapeutic constituent.

7. A tumour-targeting agent according to any one of the preceding claims in which the compound incorporates an antibody fragment capable of becoming bound to a characteristic reactive site on a tumour cell.

8. A tumour-targeting agent according to any one of the preceding claims in which the ligand comprises thyroxine or a non-functional thyroxine analogue.

9. A tumour-locating agent comprising a complex constituting a compound in accordance with any one of the preceding claims and a complementary binding protein.

10. A tumour-locating agent substantially as hereinbefore described in the Example.

11. A method of employing a tumour-targeting agent in accordance with any one of the preceding claims, in which method a quantity of the agent is introduced into the bloodstream of a living human being or animal.

12. A method according to claim 11 in which a period of time is allowed to elapse sufficient to enable at least some of any tumour cells present to become characterised by the fact that each such cell has many more molecules of the compound bound to it than there are molecules of the compound bound to an average normal cell, or the concentration of the compound bound to at least some of tumour cells is significantly greater than the concentration elsewhere in the human being or animal.

13. A method according to claim 12 in which, after that period of time, a substance is introduced into the bloodstream which competes with said compound for becoming bound to said complementary binding protein, whereby the rate at which said compound is eliminated from the bloodstream is increased.

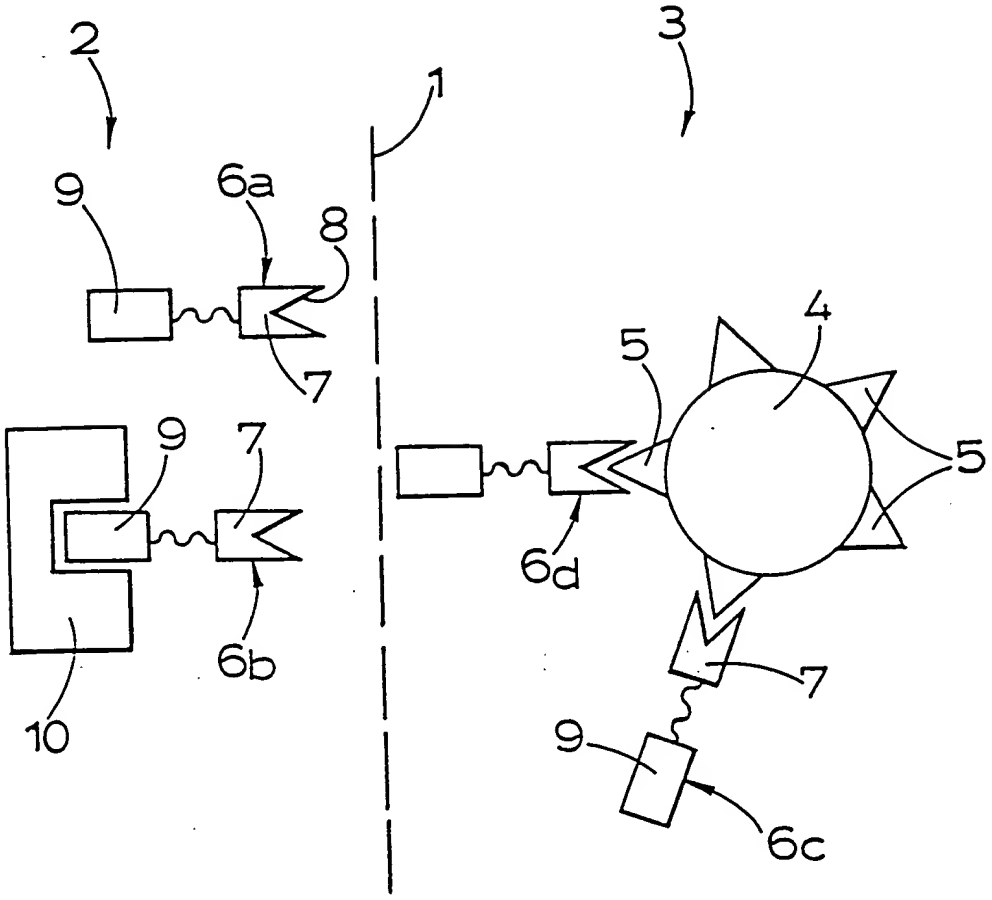

FIG.1.

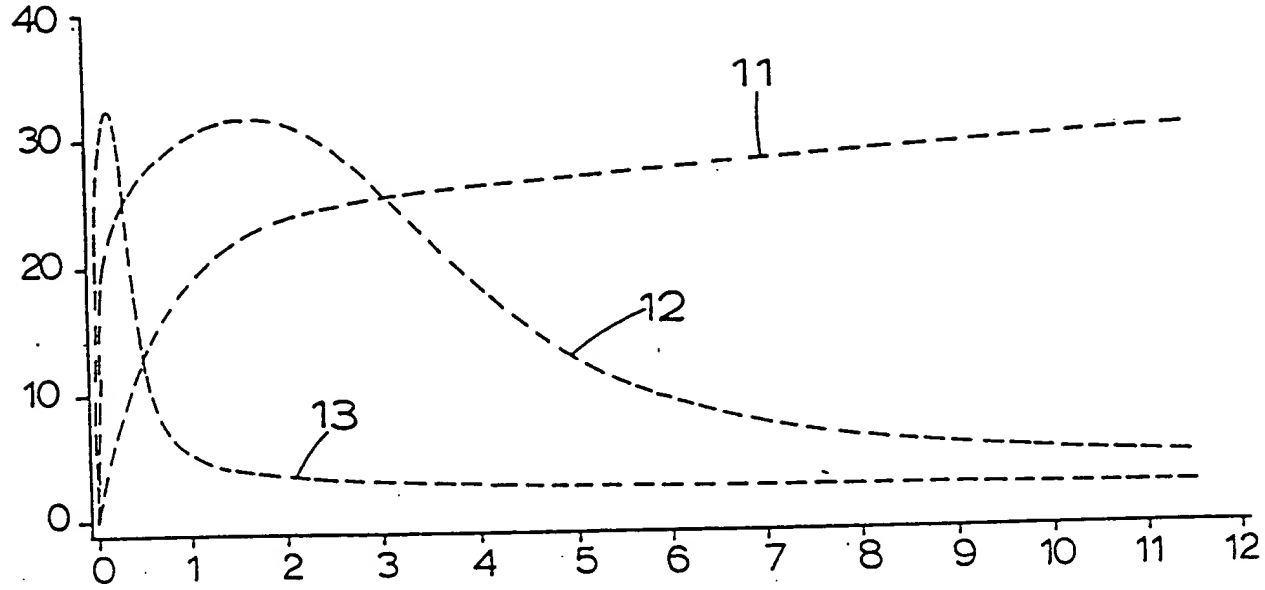
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FIG. 2.

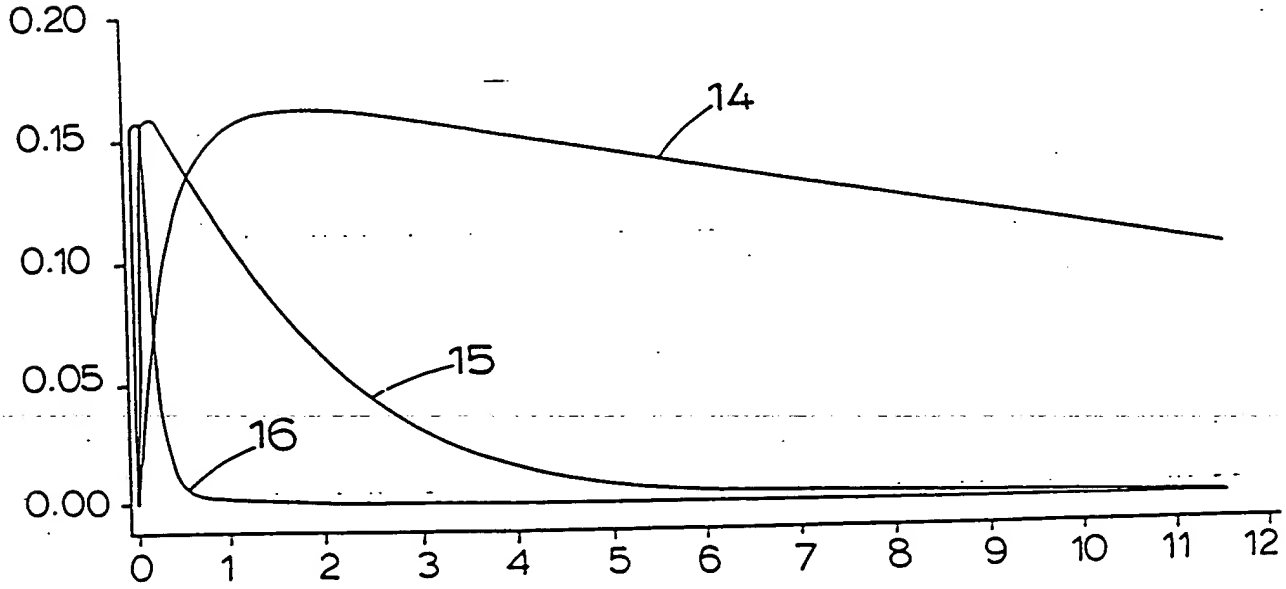
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FIG. 3.

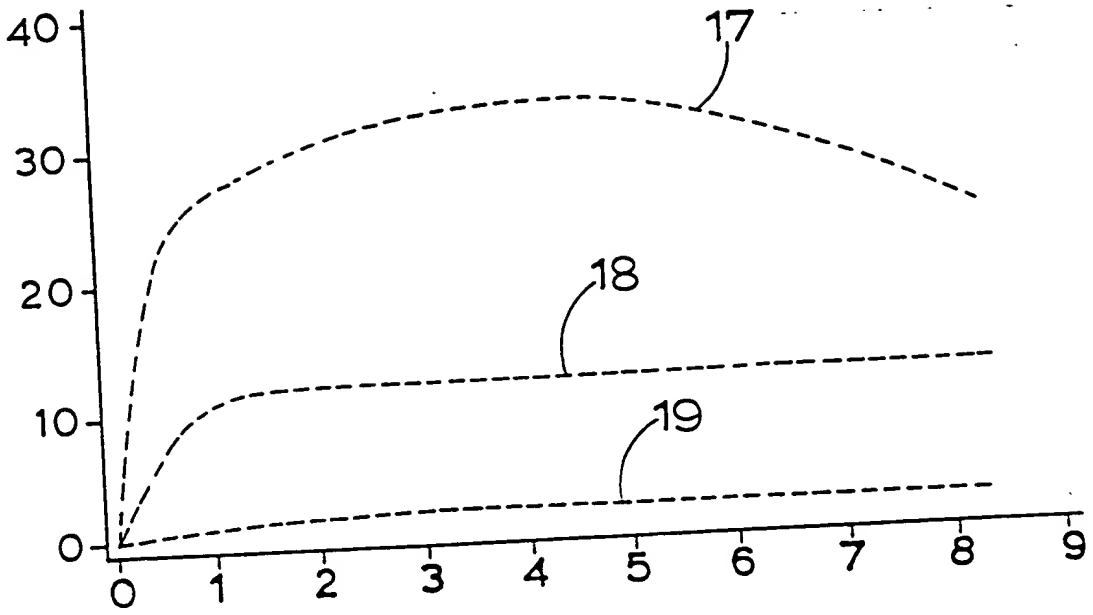
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FIG.4.

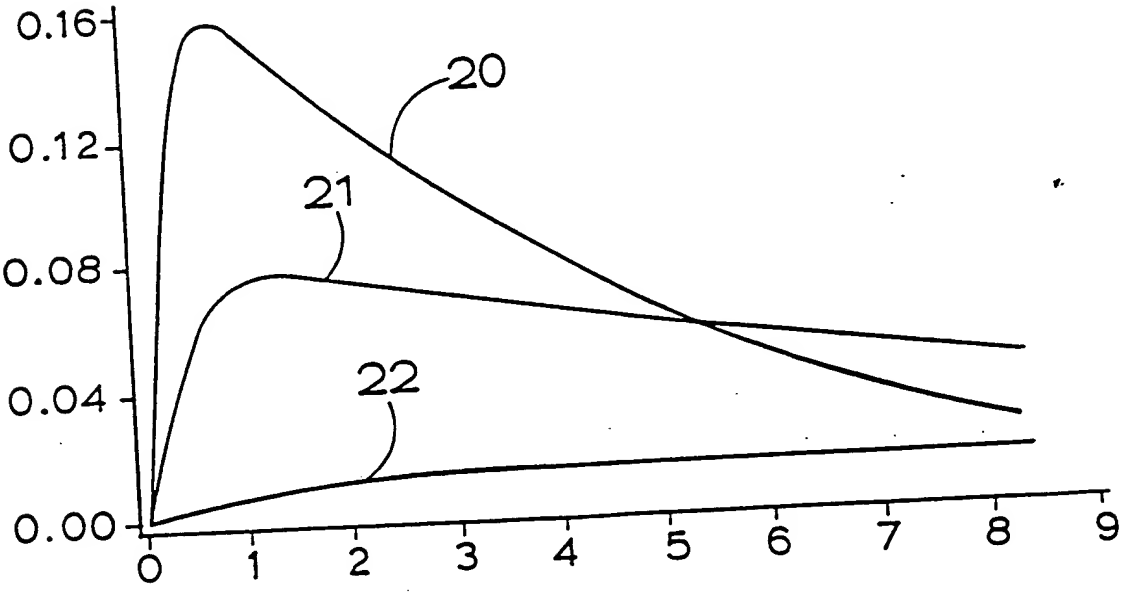
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FIG.5.

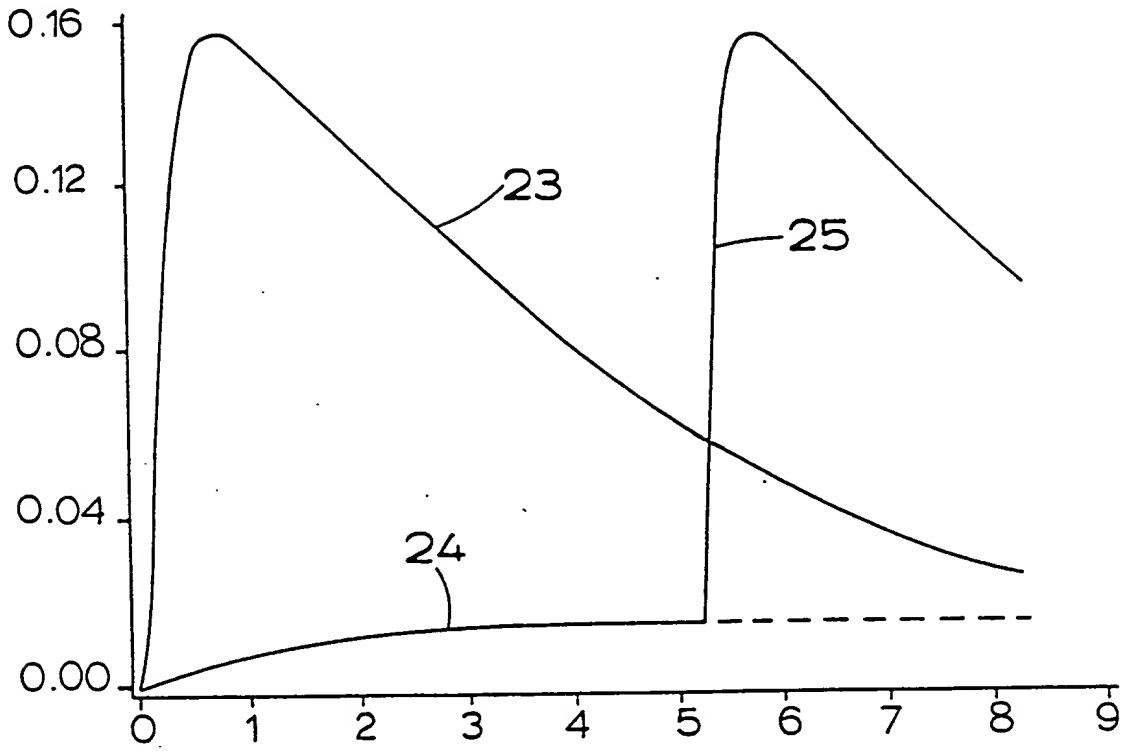

FIG. 6.

INTERNATIONAL SEARCH REPORT

PCT/GB 88/01091

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 9/18; A 61 K 47/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X,Y	FR, A, 2415301 (BAXTER) 17 August 1979, see claims 1-25; page 7, lines 9,10; page 8, lines 13-19; page 9, lines 9-12 --	1-13
Y	EP, A, 0247792 (ELI LILLY) 2 December 1987, see claims 1-10; page 9, lines 31-34; page 10, lines 21-23; page 13, lines 1-3	1-13

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28th February 1989	23 MAR 1989
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P C G. VAN DER PUTTEN

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 8801091 SA 25914

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 15/03/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-A- 2415301	17-08-79	GB-A,B 2012954	01-08-79
		DE-A- 2902400	26-07-79
		JP-A- 54104391	16-08-79
		US-A- 4271140	02-06-81
EP-A- 0247792	02-12-87	AU-A- 7334987	03-12-87
		JP-A- 62287000	12-12-87
		US-A- 4801688	31-01-89